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NADP$^+$-SPECIFIC ISOCITRATE DEHYDROGENASE INTERACTION WITH METALLOPORPHYRINS IN RAT BRAIN

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NADP$^+$-specific isocitrate dehydrogenase (IDH) in cytoplasm catalyzes the oxidative decarboxylation of isocitrate in the presence of divalent metal cations. In the mitochondria, transhydrogenase reactions are regulated by NADP$^+$-isocitrate dehydrogenase and a definite level of NAD$^+$ is maintained which is essential for NAD$^+$-isocitrate dehydrogenase functioning in the TCA cycle. We have investigated the interaction of metalloporphyrins on rat brain NADP$^+$-isocitrate dehydrogenase by subcutaneous administration of 50 μmoles/Kg b.w. of Sn-PP and PP-IX, 40 μmoles/Kg b.w. of heme and Co-PP alone and also in combination with heme to Wistar strain rats, for 2 weeks. Significantly induced isocitrate dehydrogenase activity by PP-IX and marginally induced activity by Co-PP and Sn-PP, was inhibited by simultaneous heme administration.

ABBREVIATIONS

PP-IX - protoporphyrin-IX; Sn-PP - tin-protoporphyrin; Co-PP - cobalt-protoporphyrin; Fe-PP - iron-protoporphyrin; heme or hematin.

Isocitrate dehydrogenase (IDH) [1] is the first phosphoprotein to be identified. It catalyzes the dehydrogenation of isocitrate to form an intermediate α-keto acid, oxalosuccinate which undergoes oxidative decarboxylation to 2-oxoglutarate and requires the presence of divalent metal cations.

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In most animal cells, the reaction is catalyzed by two distinct dehydrogenases, one requiring $NADP^+$ and the other having affinity for NAD^+ as co-enzyme, while the co-enzyme fragments 2′-phosphoadenosine 5′-diphosphoribose (PADPR) and 2′,5′-adenosine diphosphate (2′,5′-ADP) have been shown to function as effective competitive inhibitors with respect to $NADP^+$ [2]. In animal and plant tissues approximately 75% to 90% of the $NADP^+$-isocitrate dehydrogenase is found in the cytosol and functions in the reactions of biosynthetic processes [3–5], whereas all the NAD^+ enzyme is found in the mitochondria. In the mitochondria, $NADP^+$-isocitrate dehydrogenase participates in the regulation of the transhydrogenase reaction [4,6], which promotes the maintenance of a definite level of NAD^+, necessary for the functioning of $NADP^+$-isocitrate dehydrogenase in the tricarboxylic acid cycle. The NAD^+-isocitrate dehydrogenase is an allosteric enzyme which in animal cells is activated by ADP, which enhances its affinity for substrates [7]. In contrast, NADH inhibits isocitrate dehydrogenase by directly displacing NAD^+.

Although, some of the catalytic properties of isocitrate dehydrogenase have been studied, there is extremely little information on the active site of the enzyme. Pyridinelike nitrogen atom of imidazole is inclined to form complexes with metals – the metal-isocitrate complex (Mn^{+2}-isocitrate complex, a true substrate of NAD^+-isocitrate dehydrogenase is formed with manganese) [8–10], which confirms the possibility of formation of coordination bonds between divalent metal cations and imidazole group of histidine [11]. Because of higher affinity of metals [12] with -SH group of cysteine present in the active site of isocitrate dehydrogenase, in this communication, we report the interaction of PP-IX, Sn-PP, Co-PP and heme with isocitrate dehydrogenase in the brain of rat, when administered alone and simultaneously with heme.

EXPERIMENTAL PROCEDURES

Materials: Metalloporphyrins were purchased from Porphyrin Products (Logan, UT, USA). All other chemicals used during the present investigation were of analytical grade and purchased from Sigma Chemical Co. (MO, USA) and Pharmacia Fine Chemicals, Sweden. Female Wistar strain rats (150–200 g) were used throughout this study and housed in separate cages with raised wire mesh. They were maintained on standard rat chow and water *ad libitum*.

Treatment of Animals: Rats were injected subcutaneously [13,14] with PP-IX (50 μmoles/Kg b.w.), Co-PP (40 μmoles/Kg b.w.), Sn-PP (50 μmoles/Kg b.w.), Fe-PP or heme or hematin (40 μmoles/Kg b.w.) and/or metalloporphyrins in combination with heme. Control animals received an equivalent amount of 0.9% saline or NaCl. Fresh solutions of metallopor-phyrins were prepared in subdued light [15], immediately prior to injection by dissolving the heme and other metalloporphyrins in a small volume of 0.2N NaOH, adjusting the pH to 7.4 with 1N HCl, and making upto final volume with 0.9% saline. The purity of heme, Sn-PP, Co-PP was assessed as described previously [16].

Tissue Preparation: The animals were given access to water but were starved for 24 h after metalloporphyrin administration, prior to decapitation. Brains were removed and perfused *in situ* with ice-cold 0.9% saline. A 10% (w/v) homogenate of brain was prepared in 0.25 M sucrose solution and spun in a refrigerated centrifuge at 25,000 × g for 20 min. The supernatant was collected and assays were performed for tissue enzyme and concentartion of protein.

$NADP^+$-specific Isocitrate Dehydrogenase Activity Assay [DL-isocitrate: NADP oxidoreductase (decarboxylation) EC 1.1.1.42]: $NADP^+$-specific isocitrate dehydrogenase enzyme activity was measured by following the increase in absorbance at 340 nm as NADP gets reduced. The activity of the enzyme is calculated using NADPH extinction coefficient 6.22 $mM^{-1} cm^{-1}$ [17] and their values were expressed as nmoles of NADP reduced/min/mg protein.

Isocitrate dehydrogenase activity was assayed by the method of Ochoa [18]. Reaction mixture contained Tris-HCl, 7.5 μmoles, ph 7.4; DL-trisodium isocitrate, 0.5 μmole; MnCl, 1.8 μmoles; NADP, 0.415 μmole and 0.1 to 0.5 mg protein in a final volume of 3.0 ml. Reaction was started by the addition of NADP and followed by measuring increase in absorbance at 340 nm.

Protein Assay: Protein concentration was determined by the method of Lowry *et al.* [19] using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

To confirm the hypothesis of possible participation of histidine residue [3,11,20] and -SH group of cysteine [11,21-23] in both plants and animals,

for the functioning of $NADP^+$-isocitrate dehydrogenase, we have investigated the interaction of PP-IX and heme on isocitrate dehydrogenase activity in rat brain and also its competitive effect with Sn-PP and Co-PP. The objectives of our investigation is to study the biochemical differentiation in the parameters of body weight, brain weight and NADPH generating isocitrate dehydrogenase enzyme.

Effect of Metalloporphyrins on Body and Brain Weights: Brain starts to develop very early and its foundations and potential for further development are partially laid already at birth [24]. The change in the body and tissue (brain) weight due to administration of metalloporphyrins are given in Figs. 1 and 2. The control group which received only normal saline showed 4.9% increase in the body weight whereas increase in body weights of groups administered with metalloporphyrins alone showed heme 12.3%; PP-IX 39.6%, Co-PP 12.6% and Sn-PP 9.7%, as compared to initial weights. In contrast, there was slight decrease in body weights of animals when other metalloporphyrins were administered simultaneously with heme, for 2 weeks. The decrease in the body weights of

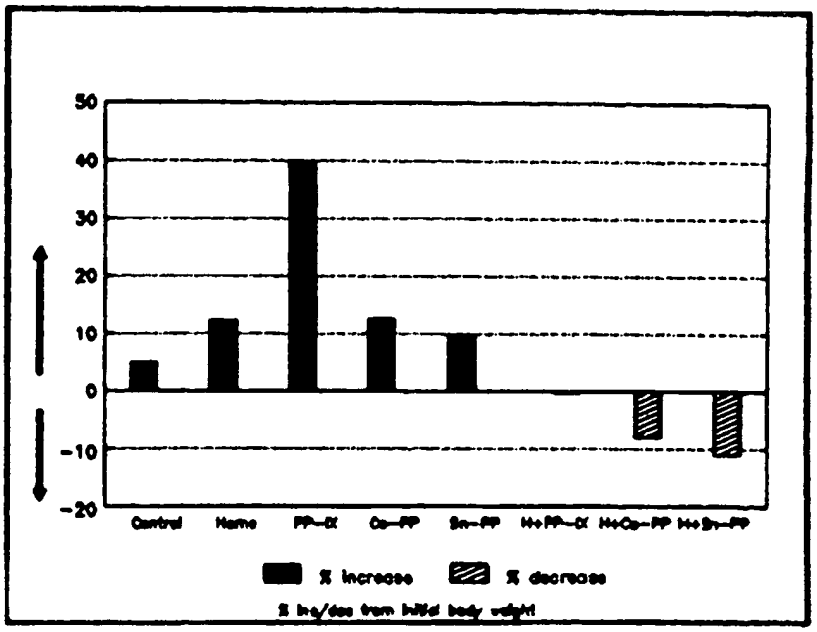

FIGURE 1 Effect of prolonged administration of PP-IX, Co-PP, Sn-PP and their combination with heme on body weight of rats, expressed as percent increase decrease from initial body weight.

treated animals was in the order, heme + PP-IX 0.35%, heme + Co-PP 7.9% and heme + Sn-PP 11.0%, as compared to control (Fig. 1).

There was marginal increase in brain weights of animals treated with metalloporphyrins alone, as compared to control. When other metalloporphyrins were administered along with heme, the tissue weight increased further in heme + PP-IX administered animals, marginally decreases by heme + Co-PP group and remained unchanged by heme + Sn-PP group, as compared to their respective controls or only metalloporphyrin administered groups (Fig. 2).

However, the tissue weights in all the groups showed increase, when compared to control (0.9% saline). This increase is PP-IX 17.1%, Co-PP 55.6%, Sn-PP 25.6%, heme 49.6%, heme + PP-IX 37.6%, heme + Co-PP 28.2%, heme + Sn-PP 25.6% (Fig. 2).

The jaw movement and teeth of experimental animals were found to be normal. The difference temporal response of brain towards metalloporphyrins administration may be attributed to the fact that the development of brain is precocious with regards to the body and other organs.

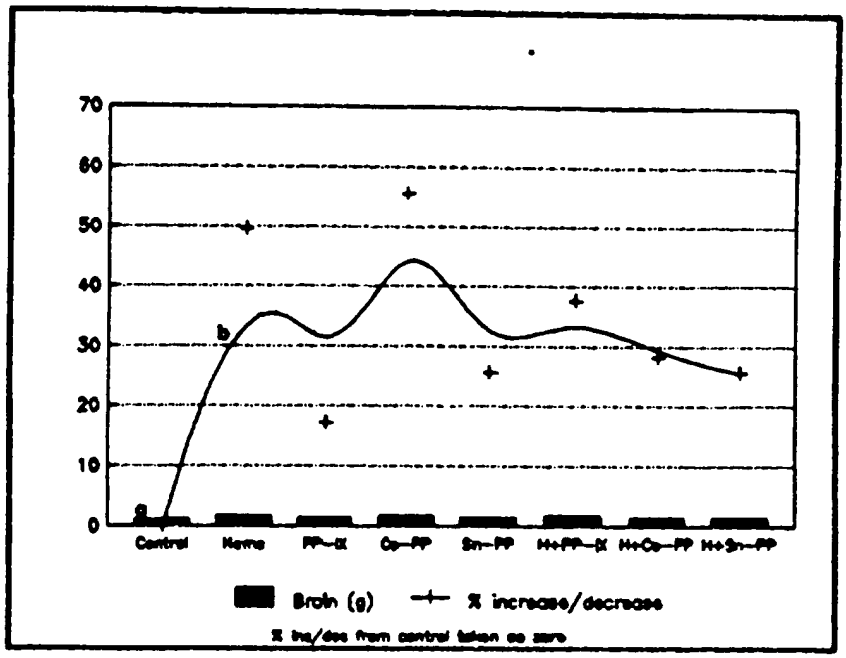

FIGURE 2 Effect of prolonged administration of PP-IX, Co-PP, Sn-PP and their combination with heme on tissue (brain) weight of rats. (a) Weight of brain in gms. (b) Percent increase-decrease of brain weight as compared to control which is taken as zero.

Interaction of Metalloporphyrins with NADP$^+$-specific Isocitrate Dehydrogenase: It has been observed that at birth, the activity of isocitrate dehydrogenase is always higher in liver as compared to brain. The activity of isocitrate dehydrogenase does not increase with age in brain, while in liver it increases with age, then it decreases and again increases, after 3 weeks. Metalloporphyrin administration affects the postnatal development of isocitrate dehydrogenase activity in brain as is given in Fig. 3. The increase in enzymic activity is significant in groups administered with PP-IX, and marginal in groups administered with heme, Co-PP and Sn-PP, as compared to control (0.9% saline). But when other metalloporphyrins were administered simultaneously with heme, the enhanced isocitrate dehydrogenase activity of brain due to administration of metalloporphyrins alone, is significantly decreased. This decrease in activity of enzyme is maximum in group administered with heme + Sn-PP, followed by heme + PP-IX and heme + Co-PP, in comparison to their respective control or metalloporphyrin administered groups (Fig. 3).

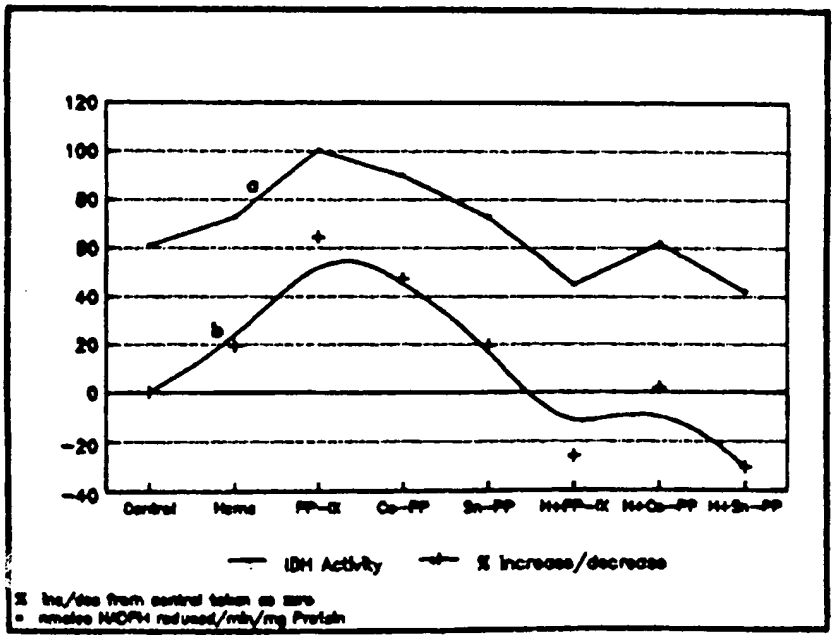

FIGURE 3 Alterations on NADP$^+$-specific isocitrate dehydrogenase activity in the brain of rat. (a) Activity of the enzyme is calculated using NADPH extinction coefficient 6.22 mM^{-1} cm^{-1} and the values are expressed as nmoles of NADP reduced/min/mg protein. (b) Activity expressed percent increase decrease as compared to control which is taken as zero.

When metalloporphyrins were administered alone, the developmental patterns of $NADP^+$-isocitrate dehydrogenase activity in brain tissues showed an increase in the brain of animals administered with PP-IX 65%, Co-PP 50%, Sn-PP 20%, heme 20%, as compared to the control (0.9% saline). In marked contrast, when other metalloporphyrins were administered in combination with heme, isocitrate dehydrogenase activity in brain tissues showed significant decrease in animals administered with heme + PP-IX 26%, heme + Sn-PP 30%, as compared to control. However, marginal increase was observed in isocitrate dehydrogenase activity in the brain of animals administered with heme + Co-PP (Fig. 3).

NADPH is an essential requirement of the living cell for its many reductive synthesis or anabolic processes and is mainly generated by the oxidative pentose phosphate. In eukaryotes, the malic enzyme and $NADP^+$-isocitrate dehydrogenase also produce NADPH [25]. The activities of malic enzyme and dehydrogenases of the pentose phosphate pathway vary concommittantly with the rates of hepatic acid syntheis over a wide range of nutritional and physiological conditions [26]. The developmental patterns of malic enzymes and isocitrate dehydrogenase has been extensively studied [27] and elucidated in liver, kidney, brain, heart and small intestines [28–30]. Dietary manipulations have been shown to affect the activities of these enzymes and NADPH-specific metabolic processes [31]. Such studies have provided an insight to the mechanism of enzyme differentiation in mammalian system and also show that the developmental pattern of these enzymes vary from organ to organ [31–33].

The reults of present investigation on the developmental patterns of the isocitrate dehydrogenases are different than those reported by Madvig and Abraham [30]. The differences appear to arise due to dietary conditions imposed by different litter size under one experimental conditions. Madvig and Abraham have used neonates whose mothers were kept on fat free diet and the neonates weaned on 17th day of age, whereas in the present study, both the neonates and mothers were kept on standard rat diet and the neonates were with their mothers till the 23rd day of age. The results of the present study on dehydrogenases in rat brain are not similar to those reported by Andres *et al.* [27,34,35]. Baquer *et al.* [36] has shown a positive correlation of isocitrate dehydrogenase, acetyl CoA carboxylase and fatty acid synthesis in brain during development which suggests that pentose phosphate pathway may have an additional role besides lipogenesis, in brain. Appel and Parrot [37] have suggested that this role could be the protection of the membrane -SH group and in the reactivity of synaptic plasma membranes.

Our results are giving two spectrums of the effect of metalloporphyrins on isocitrate dehydrogenase in rat brain: (i) When metalloporphyrins were administered alone, the activity is increased. (ii) When metalloporphyrins are administered in combination with heme, the induced activity was inhibited, which can reflect the physiological significance of the enzyme $NADP^+$-isocitrate dehydrogenase. It can, thus, be deduced from this study that according to the requirement of NADPH during development of these enzymes, levels change accordingly, and, therefore, can be used as an index of biochemical development. The effect of metalloporphyrin administration to rats during growth show that these compounds does directly or indirectly modify the developmental pattern of $NADP^+$-isocitrate dehydrogenase enzyme and thus affect the NADPH-specific metabolic processes in the cell. The mechanisms which bring about these effects are not clear. The effect of metalloporphyrin on this enzyme shows a trend of reversal at later periods of study which can be interpreted due to the metabolism of metalloporphyrins and hence, a reduction in its concentration in the cell with time.

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